



Review article

Inhibitory effect of chrysin on estrogen biosynthesis by suppression of enzyme aromatase (CYP19): A systematic review

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ABSTRACT

The cytochrome P450 enzyme functions as the rate-limiting enzyme in changing androgens to estrogens. Inhibition of aromatase is one of the significant objectives of treatment of hormone-dependent diseases such as breast cancer, especially in post-menopausal women. Natural compounds like chrysin, as a flavor that has a high concentration in honey and propolis, are rich sources of them can be useful in inhibiting aromatase for chemoprevention following treatment or in women at risk of acquiring breast cancer. This study intended to summarize the existing evidence on the effect of chrysin on aromatase activity. We systematically searched Science Direct, PubMed and Google Scholar and hand searched the reference lists of identified relevant articles, up to 5 February, 2019. Articles with English abstracts that reported the effect of chrysin on aromatase inhibition and without publication date restriction were investigated. Twenty relevant articles were chosen from a total of 1721 articles. Only one study was performed on humans and two studies were assayed on rats, while other studies were evaluated in vitro. All the studies except one showed that chrysin had the potency of aromatase inhibition; however, only one study performed on endometrial stromal cells showed that chrysin and naringenin did not indicate aromatase inhibitory properties. Various assay methods and experimental conditions were the important aspects leading to different results between the studies. Chrysin has potency in inhibition of the aromatase enzyme and thus can be useful in preventing and treating the hormone-dependent breast cancer and as an adjuvant therapy for estrogen-dependent diseases.

1. Introduction

Estrogens as steroid hormones are found in organisms in various types. Estrone and estrinol are less potent than 17 β -estradiol and thus the form responsible for many biological activities is 17 β -estradiol. The common function of estrogen is the control of sexual differentiation, puberty, and reproduction. However, estrogens are involved in controlling proliferation, cell cycle, and homeostasis of various organs. Several enzymes are involved in the process of estrogen biosynthesis [1]. Among such enzymes is the aromatase enzyme that converts androgens to estrogens. The cytochrome P450 enzyme (CYP19) (11 β -hydroxysteroid dehydrogenase) is from the cytochrome P450 family [2] that was encoded by the CYP19 gene [1]. Cancer is one of the causes of mortality and morbidity in the world, especially in the third world countries. It is

characterized with uncontrolled growth and multiplication [3]. In breast cancer, the expression of CYP19 increases and thus cell proliferation by estrogen increases [2]. Aromatase is a prime enzyme that regulates estrogen biosynthesis in local and systemic levels in the body [1]. Using aromatase inhibitor is a treatment method of breast cancer in post-menopausal women. Several synthetic compounds have been designed for inhibiting aromatase. In addition, nowadays, natural products are used for inhibition of aromatase in order to detect a novel approach for treatment of breast cancer [4].

Phytochemicals are derived from a plant with a phenolic compound being the major class of them. Phytochemicals are used to inhibit the aromatase enzyme. The aromatic ring structure is the basic structure of phenolic compounds with other elements such as oxygen atoms, methyl groups and sugars being a part of its structure. Phenolic compounds are

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classified into different groups such as flavonoids, phenolic alcohols, phenolic acids, stilbenes and lignans. The largest group of phenolic compounds with low molecular weight is flavonoids that are widely found in fruits and plants and are divided into six groups including flavanones, flavones, isoflavones, flavonols, flavanols as well as anthocyanins [5, 6] and proanthocyanidins [7]. Secondary metabolites like flavonoids function as the first line of defense against oxygen radical production, ultraviolet rays and/or invasive organisms in plant species. Diversities of flavonoid subgroups depend on a heterocyclic C ring structure binding to A and B phenolic rings with double bonds [6] and also on the degree of hydroxylation and substitutes [8].

Sources of flavonoids are fruits, vegetables, nuts, and grains, as well as various herbs and spices. Flavonoids have antibacterial, antiviral, antioxidant, anti-allergic, anti-inflammatory and anticancer [3, 9] capabilities. Chrysin (5, 7-dihydroxyflavone) is a polyphenolic flavone that has a high concentration in honey and propolis. Chrysin as an apigenin analog has the high remedial power of transferring the intestinal membrane and also has anti-inflammatory [10] and antioxidant [11] capabilities. Chrysin is reported to have the highest aromatase inhibitory potency and stimulate cell apoptosis. Thus, it can be engaged in the prevention of cancer or cancer treatment [8]. The aim of this review was to prove the claim that chrysin could inhibit aromatase and increase testosterone level.

2. Methods

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement to perform and report this systematic review [12].

2.1. Search strategy

Three databases including PubMed (www.pubmed.com), Science Direct (www.sciencedirect.com) and Google Scholar (Scholar.google.com) were researched up to 5 February, 2019. MeSH and non-MeSH terms were used as follows: ('chrysin' OR 'flavonoid') AND ('androgen' OR 'testosterone' OR 'estradiol' OR 'estrogen') AND ('mutagenesis' OR 'aromatase inhibitor'). Further, citations were known by manual marketing lists of all the selected articles and reference list of relevant articles were searched to select the eligible article. The citation was saved and managed by the EndNote X5 reference software. The process of selecting articles is shown in Figure 1.

2.2. Eligibility criteria

We included studies that reported the effect of chrysin on aromatase activity enzyme, regardless of their type, date, and outcomes. The excluded studies were narrative reviews, conference abstracts and perusals that did not report the outcomes of interest or their full texts were not available. Studies not written in English were included only if the abstract was translated into English.

2.3. Data extraction

The extracted data from the studies included first author's name, year of publication, study location, material study, sample, test method, outcomes, and main results. Study data were extracted and recorded into the summary table.

2.4. Quality assessment of studies

Risk of bias assessment of included studies was evaluated using the Science in Risk Assessment and Policy (SciRAP) in vitro reporting checklist (Table 1) [13]. The risk of bias for each included study categorizing the following items: purpose, endpoint, test compound, vehicle, ethical statement, test system, administration of test compound,

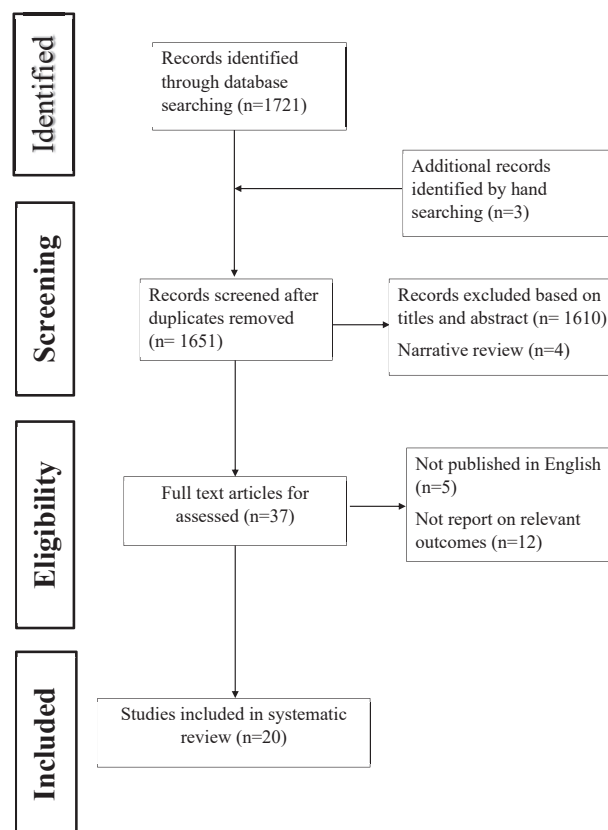


Figure 1. Process of study selection based on PRISMA flowchart.

methods, statistics, data, discussion, other. In fact, the SciRAP method evaluates both the reporting quality and the methodological quality of the studies. To evaluate the risk of bias, each study was assigned a label (fulfilled, partially fulfilled, not fulfilled and not determined). The overall quality score was judged as poor, fair and good, where score was classified as 0–5, 6–9, 10–14, respectively [14].

3. Result

Summary of extracting data from this systematic review is described in Table 2.

Kellis et al. [15] investigated the effect of flavones on aromatase, the cytochrome P450 enzyme, in human placental microsomes obtained from premenopausal women after normal deliveries. The activity of aromatase was measured by the release of tritium from (1, 2-³H) androstenedione or from (1, 2-³H) testosterone. The aromatase inhibitory power of flavones including 7, 8-benzoflavone, chrysin, apigenin, flavone, flavanone, quercetin, and 5, 6-benzoflavone decreased, respectively. When the 40 nM androstenedione was added as the substrate, 0.5 μM chrysin could inhibit 50 percent of the aromatase concentration (IC₅₀) and k_i values of chrysin were 0.26 μM. It was indicated that 7, 8-benzoflavone (α-naphthoflavone) (IC₅₀ 0.07 nM), like chrysin, acted competitively against androstenedione and was ten times as potent as aminoglutethimide. Other compounds including chrysin (IC₅₀ 0.5 nM), apigenin (IC₅₀ 1.2 nM), flavone (IC₅₀ 8 nM), flavanone (IC₅₀ 8 nM) and quercetin (IC₅₀ 12 nM) were effective, but 5, 6-benzoflavone showed no activity in this assay.

In another in vitro study, Campbell et al. [16] compared the effect of synthetic and natural flavonoids (catechin, chrysin, flavone, genistein, Biochanin A, quercetin, rutin, and daidzein and α-naphthoflavone (ANF) and β-naphthoflavone (BNF)) in aromatase inhibiting with that of aminoglutethimide (AG), a pharmaceutical aromatase suppressor, in human preadipocyte cells. This study showed that the tritiated water method

Table 1. Summary of the studies that examined the effect of chrysin on aromatase inhibitors.

First authors (year)	location	sample	Test compound	Aromatase assay	outcomes	Main result
Almstruup. K et al. (2002)	Denmark	MCF-7 cells	phytoestrogens	Modified estrogenicity assay	Aromatase activity	Aromatase inhibitors were at low concentration (<1µM), but the estrogenic response was at high concentration (>1µM)
Altawash et al. (2017)	Iran	40 week-old roosters	chrysin	Gross assessment with Burrows and Quinn method, Lipid peroxidation, Sperm fatty acid, Testosterone assay and Fertility and hatchability rates	Sperm total and forward motility, plasma membrane integrity and functionality, semen concentration, fertility and hatchability rates	Chrysin administration particularly at higher levels alleviates post-peak. It reduced fertility in the roosters. It increased blood testosterone.
Bajgai et al. (2011)	Thailand	MCF-7 cell line.	Hybrid flavon-chalcones	Based on the methodology reported by Stressor et al.	Aromatase activity	The IC ₅₀ values of chrysin was 0.8 µM
Campbell et al. (1993)	U.S.A	Subcutaneous adipose tissue from female patients aged 18-42	Natural and synthetic flavonoids	The Modified tritiated water	Aromatase activity	The IC ₅₀ values of chrysin was 4.6 µM
Dhawan et al. (2002)	India	Male Albino Rats	Chrysin (1 mg/kg) for 30 days	Sperm count and fertilization (indirect)	Aromatase activity	It raised sperm count (427*10 ⁶) and fertilization (80%)
Edmunds et al. (2005)	U.S.A	Endometrial stromal cell from 18 women aged 27–44 years	phytoestrogens	Modified tritiated water	Aromatase activity	Chrysin didn't reduce aromatase activity
Gambelunghe et al. (2003)	Italy	20 healthy male (25–30 years) (10 controls and 10 cases)	Tablets of propolis (1280mg) and honey (20mg) 21 days	Urinary testosterone levels (GC/MS analysis)	Hormonal equilibrium	It didn't change urinary testosterone.
Jeong et al. (1999)	Korea	?	Flavonoids	The tritiated water release assay	Aromatase activity	The IC ₅₀ values of chrysin was 1.1 µM
Kao et al. (1998)	United Kingdom	Chinese hamster ovary cells	phytoestrogens	The tritiated water release assay	Aromatase activity	The K _i values of chrysin was 2.6 ± 0.1 µM
Kellis et al. (1994)	Mexico	Human placental microsomes	flavones	The tritiated water release assay	Aromatase activity	The IC ₅₀ values of chrysin for 40nM androstrondion and 80nM testosterone was 0.5 and 0.4 µM, respectively
Lannone et al. (2018)	Italy	In vitro protocol (inhibition kinetics experiments monitored by GC-MS)	Flavonoids and isoflavones	Modified tritiated water	Aromatase activity	It reduced estradiol concentrations, but chrysin wwas not banned from monitoring by WADA.
Le Bail et al. (2000)	France	Human placental microsomes	phytoestrogens	Unchanged radiolabelled A4	Aromatase activity	The IC ₅₀ values of chrysin was 0.7 µM
Nega Ta and Thomas Walle (2007)	U.S.A	Methylated	methylated flavones (7-methoxyflavone, 7,4_-dimethoxyflavone and 5,7-dimethoxyflavone), chrysin	Based on the methodology reported by Stressor et al.	Aromatase activity	The IC ₅₀ values of chrysin was 4.2 µM
Pelissero et al. (1996)	France	Rainbow trout ovarian cells	phytoestrogens	The tritiated water release assay	Aromatase activity	The IC ₅₀ values of chrysin was >1000 µM and thus did not have inhibitory effect.
Saارينen at al. (2001)	Finland	JEG3 cells and Arom-HEK293 cells AND immature female rats (Sprague-Dawley)	flavonoids	The tritiated water release assay And measurement of uteri weight	Aromatase activity	The IC ₅₀ values of chrysin was lower than 1 µM And Induced Uterine growth (NS)
Sanderson et al. (2004)	California	H295R cells (human adrenocortical carcinoma cells)	Synthetic and natural flavonoids	The Modified tritiated water isolated from the culture	Aromatase activity	The IC ₅₀ values of chrysin was 7 µM
Sassa Deapaeng et al. (2017)	Thailand	Female Nile tilapia hepatic microsomes	Chrysin, quercetin, quercitrin	Based on the methodology reported by Stressor et al.	Aromatase activity	The IC ₅₀ values of chrysin was 0.25 µM
Van meeuwen et al. (2007)	Netherlands	MCF-7 cells and healthy mammary fibroblast	phytochemicals	The Modified tritiated water isolated from the culture	Aromatase activity	The IC ₅₀ values of chrysin was 1.5 µM
Van meeuwen et al. (2008)	Netherlands	Human placental microsomes and human mammary fibroblast	Synthetic lactones and flavonoids	The Modified tritiated water isolated from the culture	Aromatase activity	The IC ₅₀ values of chrysin in placental cells was 1.1 µM

The K_i value.
Chrysin content in the tablet.

Table 2. ScIRAP quality assessment of the included studies.

Study and year	Purpose and aim	Endpoints	Test compound	Vehicle	Ethical statement	Test system	Administration of test compound	Methods	Statistics	Data	Discussion	Other	overall quality
Almstrup, K et al. (2002)	fulfilled	fulfilled	not fulfilled	not determined	not determined	not fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fair
Altawash et al. (2017)	fulfilled	partially fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Bajgai et al. (2011)	partially fulfilled	partially fulfilled	not fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	partially fulfilled	fulfilled	fulfilled	fulfilled	fair
Campbell et al. (1993)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Dhawan et al. (2002)	fulfilled	fulfilled	fulfilled	not determined	not determined	partially fulfilled	fulfilled	fulfilled	partially fulfilled	fulfilled	fulfilled	fulfilled	fair
Edmunds et al. (2005)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	partially fulfilled	fulfilled	fulfilled	fulfilled	good
Gambelunghe et al. (2003)	fulfilled	fulfilled	not fulfilled	fulfilled	not determined	partially fulfilled	fulfilled	fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	fair
Jeong et al. (1999)	fulfilled	fulfilled	partially fulfilled	not determined	not determined	fulfilled	fulfilled	partially fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	good
Kao et al. (1998)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Kellis et al. (1994)	fulfilled	fulfilled	not fulfilled	not fulfilled	not determined	partially fulfilled	partially fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fair
Lannone et al. (2018)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Le Bail et al. (2000)	fulfilled	fulfilled	partially fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Nega Ta and Thomas Walle (2007)	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Pelissero et al. (1996)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	good
Saarinon et al. (2001)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	good
Sanderson et al. (2004)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	not fulfilled	fulfilled	fulfilled	fulfilled	good
Sassa Deepaeng et al. (2017)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Van meeuwen et al. (2007)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good
Van meeuwen et al. (2008)	fulfilled	fulfilled	fulfilled	fulfilled	not determined	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	fulfilled	good

measured 50 percent of the concentration of aromatase activity, which was 4.6 μM compared to aminoglutethimide ($\text{IC}_{50} = 0.5 \mu\text{M}$). Moreover, the other flavonoids did not affect the enzyme activity and K_i values of chrysin were 2.4 μM . The activity of flavone and biochanin A was marginal (IC_{50} of 68 and 113 μM , respectively). The k_m of androstenedione in the presence of 5 μM chrysin increased to 226 nM while its k_m increased to 232 nM in presence of 10 μM aminoglutethimide.

Pelissero et al carried out a study for in vitro inhibition of aromatase activity in ovarian obtained from the rainbow trout, *Oncorhynchus mykiss* [17]. Aromatase activity was assayed by the technique of De Mones and Monod et al. [18]. The study did not show any inhibitory effect of isoflavone phytoestrogens (chrysin, apigenin, quercetin, α -Naphthoflavone, flavanone, formononetin, daidzein, biochanin A, genistein, coumestrol, and equol). The reason is that the IC_{50} values of these flavonoids were over 1000 μM , which was close to 100 times higher than those reported in prior studies on mammary cells.

Computer modeling in the study of Chen et al. [19] showed how aromatase inhibitors like phytoestrogens (chrysin, 7, 8-dihydroxyflavone, baicalein, galangin, genistein, biochanin A, naringenin, and α -naphthoflavone) bound to the active site of aromatase mutants. Chrysin binds to the wild-type aromatase and six mutant active site as a competitive inhibitory enzyme regarding substrate because the A and C rings of the compounds mimic the A and B rings of steroids in the active site of aromatase. The IC_{50} values of chrysin for the P308F and D309A mutants increased due to modifying the size of the active site pocket and thus inhibited the binding of chrysin. They showed that chrysin was a stronger inhibitor than 7, 8-dihydroxyflavone with IC_{50} values of 11 and 55 μM .

Kao et al. [20] tested the effect of four flavones (chrysin, 7, 8-dihydroxyflavone, baicalein, and galangin), two isoflavones (genistein and biochanin A) and one naphthoflavone (α -naphthoflavone) on the wild-type aromatase and six mutants being expressed in Chinese hamster ovary cells. With computer modeling, the binding of phytoestrogens in the active site was performed using the program BIOGRAPH. They determined the ability of these aromatase inhibitors, according to their K_i values. The K_i values were $2.6 \pm 0.1 \mu\text{M}$ for chrysin and 10 μM and 95 μM for 7, 8-dihydroxyflavone and galangin, respectively. Computer modeling showed how the binding of phytoestrogens to the active site of aromatase was performed. Computer modeling proposed this orientation for the binding of phytoestrogens to the active site of aromatase, in which the A and C rings mimic the D and C rings of the steroid, respectively. For this reason, chrysin was the best aromatase inhibitor among these compounds.

In another study, Jeong et al. assessed the inhibitory effect of flavonoids (myricitrin, genkwanin, hyperoside, rhamnetin, tangeretin, rutin, formononetin, rhoifolin, luteolin-3', 7-diglucose, neohesperidin, flavone, and esculetin) on human placental microsomes. The result of the tritiated water release assay showed that the IC_{50} value of chrysin was 1.1 μM , which showed the higher potency of the inhibitor than hesperetin and apigenin; however, other flavonoids were not active (<50% inhibition) [21].

Le Beil et al. surveyed the estrogenic, antiestrogenic, anti-aromatase, anti- 3β -HSD and anti- 17β -HSD activity of phytoestrogens in human breast cancer cell lines (MCF-7 and MDA-MV231) and showed similar results to those of Jeong et al. This study revealed that isoflavonoids with the phenolic B ring in three positions on the pyran ring (coumestrol, genistein, daidzein, and biochanin A) had much better 3β -HSD and anti- 17β -HSD activity than the aromatase inhibitor. Flavonoids including flavone, 7-hydroxyflavone, 7-methoxyflavone, 7, 8-dihydroxyflavone, chrysin, apigenin, and naringenin suppressed the aromatase enzyme and 17β -HSD but did not inhibit the 3β -HSD enzyme. The IC_{50} values of chrysin were assessed to be 0.7 μM , which was higher than the IC_{50} values of 7-hydroxyflavone but not higher than the rest [22].

The inhibition of the aromatase enzyme was measured in human choriocarcinoma JEG3 cells and human embryonic kidney (HEK-293) cells by the aromatase expression plasmid (pUbc-AROM) process. The

effectiveness of three groups of aromatase-inhibiting flavonoids including flavone, flavonol, and flavanone (apigenin, chrysin, kaempferol, quercetin, 7-hydroxyflavone, fisetin, kaempferide, luteolin, galangin, naringenin, and pinostrobin) changed due to slight changes in their social system. The IC_{50} values of chrysin and 7-hydroxyflavone were $<1 \mu M$ ($0.5 \mu M$ and $0.35 \mu M$, respectively) whereas flavone and quercetin did not have any inhibitory effect ($IC_{50} > 100 \mu M$). In addition, immature female Sprague-Dawley rats were used to perform estrogenicity and aromatase inhibition assays. In the *in vivo* part of the study, the rats were divided into six groups and uterine weight gain was measured while the control group ($n = 7$) was injected s.c. with rape seed oil. The positive control group ($n = 9$) took 10 mg/kg MPV-2213ad p.o. and rape seed oil. In the other three groups, 50 mg/kg apigenin ($n = 4$), naringenin ($n = 5$) and chrysin ($n = 5$) were gavaged. Chrysin and naringenin had no significant effect on the weight gain of the uterus and did not significantly decreased uterine growth by androstenedione [23].

This study was conducted to assay the estrogenicity and aromatase inhibitory effects of synthetic chemicals and phytoestrogens in MCF-7 cells, simultaneously. For the estrogenicity assay, cell proliferation increasing or estrogen-induced endogenous gene initiation (expression of pS2 mRNA) was investigated increasing. Potential aromatase inhibitors of the tested compounds (4-hydroxy-4androstene-3, 17-dione, anastrozole, biochanin A, genistein, formononetin, naringenin, chrysin, red clover flowers (dried powder, suspended in 10% (v/w) in ethanol), nonylphenol, bisphenol A, dibutyl phthalate, and dieldrin) assessed when exposed to testosterone pS2 mRNA expression level decreased dose-dependently. In this study, it was shown that by adding 100 nM testosterone to the culture medium, the expression level of pS2 mRNA was reduced at low concentration ($<1 \mu M$) but estrogenicity of the compounds increased at high concentration ($>1 \mu M$). The combination of aromatase inhibition and estrogenicity led to U-shaped dose-response curves. Biochanin A followed by naringenin and chrysin had the high potency of an aromatase inhibitor, respectively [24].

Dhawan et al. [25] described the potential beneficial effects of iso-flavones to counteract the adverse effects of aging on sexual male mice behavior. To this end, male albino rats of Wistar strain were divided into three groups ($n = 10$ in all the groups). The control group received vehicle (simple syrup and CMC) while one treatment group received 1 mg/kg chrysin suspended in the vehicle and the other treatment group received orally 10 mg/kg benzoflavone moiety (BZF) suspended in the vehicle for 30 days. In both the treatment groups, sexual functions such as libido, fertilization potential, sperm count and litter size increased when they were permitted to interact with proven proestrous female rats of a similar breed. In addition, the treatment groups revealed an increase in libido when they were permitted to interact with proven nonestrous mice. It appears that BZF had the higher aromatase inhibitor effect than chrysin (sperm count was 428×10^6 for BZF and 427×10^6 for chrysin).

Gambelunghe et al. performed a study to examine the effect of chrysin on urinary testosterone levels in human males aged 25–30 years. Since propolis and honey are rich sources of chrysin (54 mg/g in propolis and 1 mg/g in honey), 20 gr honey and four tablets of propolis (1280mg) were administered to healthy males for 21 days, which were consumed every morning on an empty stomach. Urinary testosterone levels were analyzed using the GC/MS method. The results revealed that urinary testosterone levels of 10 males in the treatment group did not change in comparison with baseline levels in control groups. They expected chrysin consumption to result in aromatase inhibition and increased urinary testosterone levels; however, the results did not show such effects [26].

Sanderson et al. [27] surveyed the effect of natural and synthetic flavonoids on aromatase inhibition in H295R human adrenocortical carcinoma cells. In this study, aromatase activity was assayed by the method of Lephart and Simpson (1991). The study showed that the IC_{50} values of 7-Hydroxyflavone, chrysin, and apigenin were 4, 7 and 20 μM , respectively. In addition, the study for the first time evaluated the aromatase activity of rotenone as a flavone (IC_{50} of 0.3 μM) after 24 h of exposure.

In another study, the effect of dietary phytoestrogens was assessed on CYP19 activity in human endometrial stromal cells by Edmund et al. In this study, they used a radiometric technique that measures the incorporation of tritium from (1β - 3H) androstenedione into 3H -labeled water for evaluating the aromatase inhibitory effect of phytoestrogens. The results showed that naringenin ($K_i = 0.3 \mu M$) and chrysin ($K_i = 1 \mu M$) had higher aromatase inhibition potency than genistein and daidzein ($K_i > 50 \mu M$). Genistein resulted in significantly increased aromatase activity ($P < 0.05$) whereas naringenin and chrysin inhibited aromatase activity in the recombinant human aromatase assay [28].

In the study of Nga Ta and Thomas Walle, it was revealed that methylated flavones such as 5,7-dimethoxyflavone, 7-methoxyflavone and 7,4-dimethoxyflavone were more resistant to metabolism than unmethylated analogs and thus they could inhibit the aromatase enzyme more efficiently. Therefore, they were investigated using recombinant CYP19 supersomes as the source of enzymes and dibenzylfluorescein as the substrate in a 96-well format. Although 5,7-dimethoxyflavone was the methylated form of chrysin, its effect was lower than chrysin [29].

Van meenuwen et al. examined the estrogenicity and aromatase inhibitory effect of phytochemicals (biochanin A, genistein, naringenin, apigenin, and chrysin) in two separate cell types, i.e. human breast adenocarcinoma MCF-7 cells and primary fibroblasts from healthy mammary tissues. An aromatase assay was performed using the method of Lephart and Simpson. Initially, investigating the effect of dexamethasone (DEX) as an aromatase inducer in the fibroblasts showed that incubation with 30 nM DEX increased after five weeks compared to a seven-week culturing period. However, no significant effect was observed in MCF-7 cells. Phytochemicals in the MCF-7 cells stimulated cell proliferation where the EC_{50} value of chrysin was 4 μM , which was lower than that of the other phytochemicals. Thus, the proliferation potency of chrysin had the minimum efficacy whereas the proliferation potency of biochanin A, genistein, and naringenin had the maximum efficacy. Although the proliferation potency of chrysin had the least aromatase inhibitory effect, it was more potent than the other phytochemicals. At the concentration of $>1 \mu M$ for chrysin and naringenin and $>10 \mu M$ for apigenin, the aromatase enzyme was significantly inhibited, if the concentrations of 30 μM and 100 μM for chrysin and 100 μM for apigenin were the cytotoxicity effect when measured with LDH assay after 24 h. The IC_{50} value of chrysin was 1.5 μM with more potency for aromatase inhibition whereas quercetin with $IC_{50} = 30 \mu M$ was the least potent for aromatase inhibition. Moreover, assessing the effect of phytochemicals in the co-culture of the both cell types showed that pS2 expression as a marker for measuring ER instead of cell proliferation of the MCF-7 cells increased in response to androstenedione and testosterone. Biochanin A, naringenin and chrysin up-regulated pS2 expression without testosterone at the same concentration is currently similar to that required for cell proliferation of the MCF-7 cells only. In the presence of 20 nM testosterone, chrysin did not inhibit the aromatase enzyme at an estrogenic concentration [30].

In a comparative study performed by Van Meeuwen et al. in 2008, the aromatase inhibitory effect of fadrozole (FAD), 8-prenylnaringenin and a synthetic lactone (TM-7) was investigated on both human placental microsomes and human breast fibroblasts. Apigenin (APG), chrysin, naringenin and two synthetic lactones (TM-8 and TM-9) were also examined in human placental microsomes from a healthy woman only. After incubation with dexamethasone, the mean aromatase activity was 2346 ± 307 pmol/h/mg protein in the microsomes while it was 4.3 ± 1 pmol/h/mg protein in human breast cancer fibroblasts. In placental microsomes 1 μM , 4-OH-androstenedione reduced aromatase activity by 5%. However, 4-OH-androstenedione was significantly higher than FAD at 0.1 μM . The degree of TM-7 (187.0 ± 18.7), 8-PN (159.2 ± 43.5) and APG (170.6 ± 16.4) inhibited the aromatase enzyme similar to 4-OH A, but at high concentration. However, chrysin inhibited aromatase activity with the mean of 199.5 ± 29.0 , which showed higher potency in aromatase inhibition compared to naringenin, apigenin, and TM-7. The IC_{50} values of

the investigated compounds were in the range of 0.2–13 μM , while FAD was about 4 nM [31].

Bajgai et al. examined the hybrid flavan-chalcones, desmosflavans A and B with cardamonin, pinocembrin and chrysin separated from leaves of *Desmos cochinchinensis*. The investigated compounds were also evaluated for invariant types of cell lines in order to assess cytotoxicity properties, lipoxygenase inhibition, and inhibition of superoxide anion radical formation by the xanthine/xanthine oxidase (XXO assay) and aromatase activity assay according to the method reported by Stresser et al. None of the assessed compounds scavenged superoxide anion radicals. However, cytotoxic activity toward HuCCA-1, HepG2, A549, and MOLT-3 cancer cell lines with the IC_{50} values of chrysin for the XXO assay was 79.8 μM . The IC_{50} value of chrysin aromatase inhibition was 0.8 μM ; while cardamonin was inactive, desmosflavans A and B and pinocembrin were the aromatase inhibitors with IC_{50} values of 1.8, 3.3 and 0.9 μM , respectively [32].

The report of Altawashs et al. showed the effect of chrysin on the sperm quality, fertility, fatty acid composition, hatchability rates and blood testosterone levels of 28 sexually mature Ross 308 roosters for 12 weeks. The roosters were divided into four groups and consumed encapsulated chrysin in doses of 0 (Ch0), 25 (Ch25), 50 (Ch50) or 75 (Ch75) mg/bird/day. Body sampling, semen, and blood sampling were carried out every week. All the three artificial inoculations were performed in the last two weeks of the experiment. In addition to body weight, total and forward sperm mortality were reduced by 7% in both the Ch50 and Ch75 groups. The interaction of time and treatment for sperm total and forward mortality, plasma membrane functionality, and integrity was significant. In both the Ch50 and Ch75 groups, plasma membrane functionality (71.41 and 70.2, respectively) and integrity (83.42 and 84.42, respectively) increased. Moreover, the seminal MDA content decreased significantly, but was not significant between the groups. However, blood testosterone levels increased by 5% only in the Ch75 group. When the semen sample was calculated in the 12th week, the fatty acid profile of the sperm plasma membrane was assessed. This assessment showed that the total saturated fatty acid reduced from 45.49 in the Ch0 group to 41.99, 40.29 and 39.89 in the Ch25, Ch50, and Ch75 groups, respectively. The fertility and hatchability rates increased by 7 and 6%, respectively, and became 87.4 and 86 for the Ch50 and Ch75 groups as compared to 81.2 in the control group. Finally, in the 12th week, all the roosters were slaughtered and their testicles were removed, attentively. The testis index was not significantly different among the treated groups [33].

Iannone et al. probed the effects of two main synthetic isoflavones, namely ipriflavone and methoxyisoflavone, on the catalytic activity of CYP19 and compared them with natural flavonoids (chrysin, quercetin, and daidzein) and synthetic aromatase inhibitors already considered in the WADA prohibited list (formestane, anastrozole, and aminoglutethimide, included in the section S4 of hormones and metabolic modulators). Due to the incubation of various concentrations of synthetic aromatase inhibitors (0.4, 0.8, and 1.2 μM) with 1 μM of the substrate (testosterone), a decrease was indicated in the effect on CYP19. The incubation of natural flavonoids according to their IC_{25} , IC_{50} , and IC_{75} (formestane, anastrozole, and aminoglutethimide 0.4, 0.8, and 1.2 μM , respectively; daidzein 1, 5, and 10 μM ; chrysin 0.35, 0.7, and 1 μM ; and quercetin 25, 50, and 100 μM) led to decreased aromatase activity, competitively. After the incubation of methoxyisoflavone and ipriflavone at different concentrations (5, 10, and 50 μM), they decreased the conversion of testosterone to estradiol. The K_i values of ipriflavone and methoxyisoflavone were 0.19 μM and 0.36 μM , respectively. Thus, two synthetic isoflavones were competitive aromatase inhibitors [5].

4. Discussion

The aim of this review was to prove this claim that chrysin as a flavonoid can inhibit the aromatase enzyme. Most of the studies showed the inhibition effect of chrysin on aromatase activity. Aromatase, i.e.

cytochrome P450, catalyzed the biosynthesis of estrogen from testosterone excessive estrogen and had a major effect on cell proliferation and cell cycle. Abnormal aromatase expression plays an important role in increasing breast cancer cells and endometriosis [1, 28]. In addition, in men, estrogen could contract seminiferous tubules and might have mitogenic effects on Sertoli and Leydig cells [26]. In the reviewed studies, in addition to chrysin, other phytochemicals such as isoflavonoids, flavonones, as another subgroup of flavone, and even synthetic flavonoids were assessed for their aromatase inhibitory potency. Chrysin is a dihydroxyflavone, in which two groups of hydroxyl are located at the positions 5 and 7 of the A ring [9]. According to the literature, flavones had higher aromatase inhibition potency than flavanones, isoflavones and isoflavones, respectively. Although the test of aromatase inhibitory features of flavonoids has not been performed on human populations, a mechanism is indicated, in which flavonoids can be applied to prevent or treat cancers [9, 34]. Probably the A and C rings of the activity site of aromatase mimic the D and C ring of the substrate [20]. Thus, flavones might compete with steroids to bind to the active site of aromatase. Chrysin is the best aromatase inhibitor among flavones since the hydroxyl group at the position 7 is the essential factor for inhibition [23, 35]. The 4-keto group of the C ring led to enhanced inhibitory potency of flavonoids [36, 37]. Because of being the hydroxyl group at the 5 position in the A ring (chrysin), its inhibitory potency was lower than 7-hydroxyflavone due to the formation of a hydrogen bond with the 4-keto group [20, 35, 36, 37]. The 4-keto group of chrysin pointed toward the heme prosthetic group, but C5 hydroxyl reduced the interaction of chrysin with the heme [20, 38, 39]. Likewise, a model clarified why some polyphenols such as epicatechin and epigallocatechin did not have an aromatase inhibitory feature, due to lack of the keto group [20]. Reduction of the carbonyl group to 4-hydroxyl caused the decrease of inhibitory potency like flavone-4-ol ($\text{IC}_{50} = 120 \mu\text{M}$) [35]. Apigenin and naringenin can inhibit aromatase at the 1 μM concentration and thus their aromatase inhibition potency is lower than chrysin due to being the hydroxyl group at the three positions of flavone [40]. Moreover, even the inhibitory potency of the inhibition of naringenin was less than that of apigenin because of changing the C2 and C3 double bond to a single bond [40]. The single bond between C2 and C3 in naringenin, by reducing the electronegativity of the 4-keto group, prohibited the interplay of this group with the prosthetic group [20, 23]. Quercetin, quercetin, and chrysin aromatase inhibitory potency increased, respectively [15, 41]. The core structure of the three compounds was the same but quercetin had two hydroxyl groups at the C3' and C4' positions [42], and adding the glycoside formed from deoxyribose substitution at C3 to quercetin yielded quercitrin [38]. Therefore, the structure of the compounds was related to the activity of aromatase with regard to the hydrophobic of the active site of aromatase, the substitution of the OH group or sugar led to decreased hydrophobicity of chrysin and lowered its potential anti-aromatase activity [41]. The 4', 5, 7-trihydroxy-8-prenylnaringenin (8-PN) is a prenylflavonoid where the prenyl group is located on C8 and its potential anti-aromatase is higher than that of chrysin [31, 43]. The prenyl group, in contrast to the hydroxyl group, at the C8 position in 7, 8-dihydroxyflavone [20] led to increased potency of anti-aromatase activity. The IC_{50} values of chrysin in reviewing studies were between the range of 0.5 and 5 μM . The condition of the experiment is among the essential parameters that can create different results. Conditions used for incubation, amounts of proteins incubated, pre-incubation time and conditions, incubation time and the sample used are conditional parameters that may have an effect on the degree of inhibition and IC_{50} values [17, 21, 27, 28]. For example, naringenin and chrysin were ineffective on aromatase inhibition in endometrial stromal cells while genistein induced increased aromatase activity. However, these compounds are beneficial in breast cancer.

The low oral bioavailability was because of the release of the hydrolyzing enzyme and interaction with another substrate in the gastrointestinal tract [26]. Humans' diet contains several flavonoids at different concentrations and thus it is difficult to measure the net effect of one

component such as chrysin on aromatase activity. The mixture of flavonoids in foods may have an inhibitory or inductive effect on the aromatase enzyme or another enzyme that have a role in antioxidation, anti-inflammatory, etc. Therefore, it is difficult to determine the concentration of flavonoids in human blood plasma. Since various factors affect flavonoids (e.g., chrysin) performance, consumption of single flavonoid supplementation can sufficiently increase the tissue concentration of single flavonoid to inhibit the aromatase enzyme. The drastically decreased biosynthesis of the induced estrogens induces disrupt in the menstrual cycle in females [44], impaired spermatogenesis [45] and disrupted bone homeostasis in both genders [46].

In brief, the 4-keto group in the C ring appeared to be necessary for inhibiting the aromatase enzyme. In that event, compounds such as catechins or anthocyanidins, due to the lack of the 4-keto group, cannot inhibit aromatase [47]. The presence of the 7-hydroxy or 7, 8-benzo group on the A ring enhanced aromatase inhibition. Big substitutions on the 5- or 6-position of the A ring as well as substitutions on the 3-position of the C ring can reduce the electronegativity of the 4-keto group and decrease aromatase activity, ultimately. In addition, the existence of electron donating groups on the B ring improves the potency of aromatase inhibition, however, the above constructions are not essential for anti-aromatase activity.

5. Conclusion

In conclusion, the results of this study suggest that dietary compounds, such as chrysin, which are found in foods such as honey and propolis can reduce estrogen production. Chrysin is an almost strong aromatase inhibitor in a subgroup of phytochemicals, which can inhibit the proliferation of cancer cells as well. Since much of the research is performed in vitro, in vivo studies are required to evaluate the aromatase inhibition potency of chrysin.

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